biosynthesis in the two tissues as shown previously[5,7] but also to its specific effect on protein catabolism in the latter.

Résumé. Les protéines des tissus de rats ont été marquées au moyen de L-Lysine-4, 5-H3. Une inoculation de Carcinome Walker 256 a été effectuée 7 jours après. Des observations de perte de protéines radioactives indiquent que chez les rats avec tumeur la dégradation des protéines du gastrocnémius augmente celle des protéines du foie, diminue et que celle du soléus n'est pas modifiée.

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[7] G. A. J. GOODLAD and C. M. CLARK, Eur. J. Cancer *8*, 647 (1972).

[8] This work was supported by the Scottish Hospital Endowments Research Trust.

Platelet Aggregation by Thrombofax®. Studies on the Mechanism of Action[1]

We have reported in a previous communication[2] the platelet-aggregating activity of Thrombofax (Ortho), a commercial cephalin prepared as an ether extract of acetone-dried brain[3]. This reagent is ready-to-use, stable and gives highly reproducible results. It has been shown to aggregate platelets from humans[2], dogs, guinea-pigs and rats[4].

The addition of 0.2 ml Thrombofax to 0.8 ml stirred normal citrated platelet-rich plasma (PRP, 300,000 platelets/µl) in a Born aggregometer provokes a strong platelet aggregation after a latent period; the duration of the latent period is inversely, and the initial rate of aggregation is directly, related to the amount of Thrombofax added (Figure 1). A continuous stirring of the system is required to obtain platelet aggregation. Previous incubation of PRP with Thrombofax at 37 °C, before stirring is started, shortens or abolishes the latent period before aggregation (Figure 2). In the presence of non-aggregating amounts of Thrombofax, a strong second wave of aggregation appears following addition of either adenosine-5′-diphosphate (ADP) (Figure 3) or adrenaline (Figure 4) at concentrations unable to provoke by themselves a second wave of aggregation, indicating synergism of Thrombofax with these aggregating agents. Recently it has been shown[5] that Thrombofax, like other aggregating agents, induces retraction in PRP clotted by reptilase.

The possible role of thrombin in mediating the aggregating activity of Thrombofax could be excluded by the observation that concentrations of heparin, which inhibit the aggregating activity of at least 130 N.I.H. units/ml thrombin, are ineffective on aggregation by Thrombofax; in addition, formation of plasma clots during or after aggregation by Thrombofax was never observed; finally, platelets from patients with congenital deficiency of factor V, VII or VIII reacted normally to Thrombofax.

Thrombofax preincubated at 37 °C with 8 mg/ml human albumin (Hyland) progressively loses its aggregating activity which usually disappears within 30–45 min. Preincubation of Thrombofax with normal human platelet-poor plasma does not modify its aggregating properties. Since it has been demonstrated by WARNER et al.[6] that albumin inhibits the aggregating property of free fatty acids (FFA), it is likely that FFA, which indeed constitute about $^1/_3$ of the total lipid content of Thrombofax[7], are responsible for its aggregating activity. The aggregating activity of Thrombofax was not linked to the thermolabile fraction in this reagent, which precipitates at 56 °C. Platelet aggregation by phospholipids and FFA has been reported by several authors[6,8–10]. HASLAM[9] demonstrated that the aggregating effect of FFA was mediated by the release of endogenous platelet ADP.

That Thrombofax induces platelet aggregation by a similar mechanism is supported by the following observa-

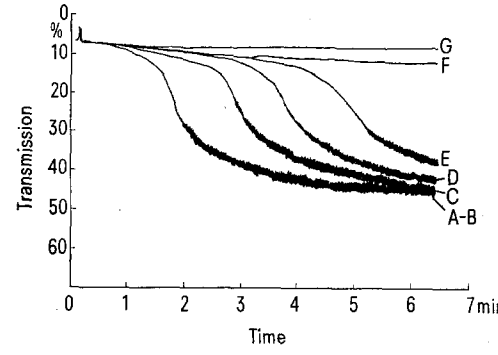

Fig. 1. Human platelet aggregation by different concentrations of Thrombofax. To 0.8 ml PRP were added 0.2 ml of Thrombofax at the following dilutions (in isotonic saline): A, undiluted; B, dilution × 2; C, dilution × 4; D, dilution × 8; E, dilution × 16; F, dilution × 32; G, dilution × 64.

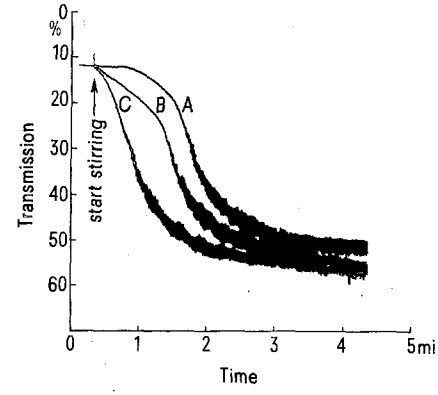

Fig. 2. Effect of previous incubation of human PRP with Thrombofax at 37 °C, without stirring, on subsequent platelet aggregation. A, no preincubation; B, 30 sec preincubation; C, 60 sec preincubation.

[1] Supported in part by grant No. 1216 of the Fonds voor Geneeskundig Wetenschappelijk Onderzoek, Brussels.

[2] G. DE GAETANO, A. VANDENBUSSCHE and J. VERMYLEN, Experientia *28*, 1127 (1972).

[3] B. BAKER, personal communication (1969).

[4] F. DECLERCK, personal communication (1971).

[5] G. DE GAETANO, D. BOTTECCHIA and J. VERMYLEN, Thrombosis Res. *2*, 71 (1973).

[6] E. D. WARNER, J. C. HOAK and W. C. CONNOR, in *Platelets: Their Role in Hemostasis and Thrombosis* (Schattauer, Stuttgart 1967), p. 249.

[7] C. M. VAN GENT, personal communication (1969).

[8] P. A. SHORE and H. S. ALPERS, Nature, Lond. *200*, 1331 (1963).

[9] R. J. HASLAM, Nature, Lond. *202*, 765 (1964).

[10] J. W. KERR, P. PIRRIE, J. MACAULAY and B. BRONTE-STEWART, Lancet *1*, 1296 (1965).

tions: 1. After platelet aggregation by Thrombofax has occurred, the sample is rapidly centrifuged to remove aggregates; then 0.2 ml of the supernatant is added to 0.8 ml PRP in the aggregometer: an immediate aggregation is invariably obtained, indicating the presence of an aggregating activity other than Thrombofax; this activity was found to correspond to about 1 μM ADP/10^8 platelets, measured as platelet-aggregating equivalent, according to WEISS and ROGERS[11]. 2. Adenosine, prostaglandin E_1, EDTA, apyrase, the enzymatic system phosphoenolpyruvate-pyruvate kinase, 2-deoxy-D-glucose and antimycin A (the last 2 substances used simultaneously) all inhibit platelet aggregation by Thrombofax at the same concentrations which inhibit aggregation by 2 μM ADP. 3. Both acetylsalicylic acid and indomethacin, at concentrations inhibiting platelet aggregation by 40 µg/ml collagen (Stago), strongly inhibit aggregation by Thrombofax. The aggregation by Thrombofax was also inhibited in plasma from normal people receiving the above anti-inflammatory drugs[12,13].

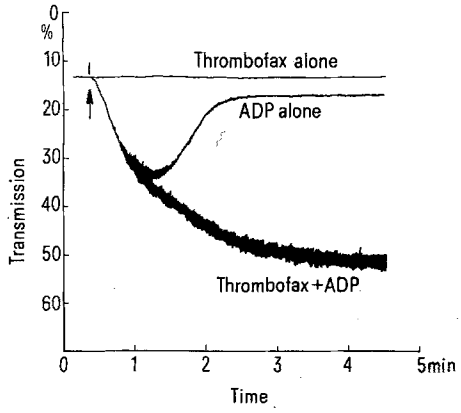

Fig. 3. Effect of a non-aggregating concentration of Thrombofax (diluted × 64 in isotonic saline) on human platelet aggregation by ADP ($2 \times 10^{-7} M$, final concentration).

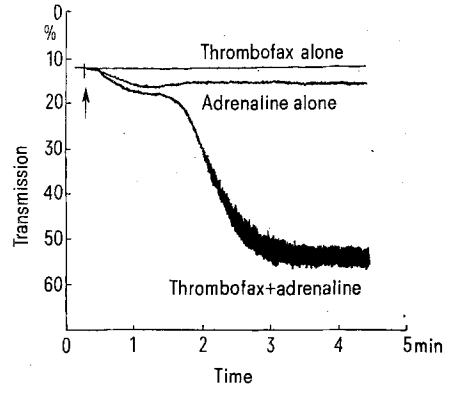

Fig. 4. Effect of a non-aggregating concentration of Thrombofax (diluted × 64 in isotonic saline) on human platelet aggregation by adrenaline ($10^{-6} M$, final concentration).

That Thrombofax induces the platelet 'release reaction' is also suggested by the following observations: 1. Thrombofax releases ^{14}C-serotonin from normal platelets: after 10 min aggregation, between 60% and 80% of the platelet-bound ^{14}C-serotonin is extruded. Acetylsalicylic acid and indomethacin ($2 \times 10^{-4} M$ final concentration) almost totally inhibit such a release. 2. Thrombofax also provokes the release of platelet factor-4 (PF-4) activity, measured by the method of HARADA and ZUCKER[14] as described by DONATI et al.[15].

In 12 normal subjects, the mean PF-4 activity released after 10 min aggregation by Thrombofax was 0.46 ± 0.08 PF-4 units/ml, which corresponds to about 80% of the mean total PF-4 activity (obtained by incubating PRP with Triton X-100[15]). Release of PF-4 activity, as well as aggregation by Thrombofax, are less pronounced at room temperature than at 37 °C. Acetylsalicylic acid and indomethacin ($2 \times 10^{-4} M$, final concentration) strongly inhibit the release of PF-4 induced by Thrombofax. The ingestion of 500 mg acetylsalicylic acid or 50 mg indomethacin provokes the same effect.

In conclusion, Thrombofax brings about platelet aggregation through the release of endogenous platelet ADP; ^{14}C-serotonin and PF-4 activity are released at the same time. FFA contained in the reagent seem to be responsible for the observed phenomena[16].

Résumé. Le Thrombofax Ortho provoque l'agrégation des plaquettes humaines (ainsi que celles du chien, du cobaye et du rat) suite à la libération d'ADP endoplaquettaire. La ^{14}C-sérotonine et le facteur plaquettaire 4 sont aussi libérés au cours de la réaction. La fraction active du produit semble résider dans les acides gras libres.

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[11] H. J. WEISS and J. ROGERS, Blood *39*, 187 (1972).
[12] G. DE GAETANO, M. B. DONATI and J. VERMYLEN, Int. J. clin. Pharmac. *5*, 196 (1971).
[13] G. DE GAETANO, M. CASTEELS-VAN DAELE, J. H. CLAES and R. EECKELS, Helv. paediat. Acta *26*, 423 (1971).
[14] K. HARADA and M. B. ZUCKER, Thromb. Diath. haemorrh. *25*, 41 (1971).
[15] M. B. DONATI, M. PALESTER-CHLEBOWCZYK, G. DE GAETANO and J. VERMYLEN, Adv. expt. Med. Biol. *34*, 295 (1972).
[16] The experienced technical assistance of Miss A. VANDENBUSSCHE is gratefully acknowledged.
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Effect of Phytohaemagglutinin on the Course of Lymphocytic Choriomeningitis Virus Infection in Mice

The effect of phytohaemagglutinin (PHA) on different types of cellular immune response has been investigated by several authors. However, experimental data concerning the influence of PHA on the cellular immune response seem to be contradictory. This effect of PHA depends on the mitogenic activity of the preparation as well as on the dose and mode of its application[1,2]. The course of intracerebral (i.cer.) lymphocytic choriomeningitis (LCM) virus infection has been studied in our earlier experiments

[1] K. MARKLEY, S. W. THORTON and E. SMALLMAN, Proc. Soc. exp. Biol. Med. *139*, 37 (1972).
[2] GY. JÁNOSSY, GY. PETRÁNYI JR., and P. ALFÖLDY, Folia biol., Praha *15*, 453 (1969).